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HEREDITY OF TRICOLOR IN GUINEA-PIGS

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WE undertook the following experiments with guinea-pigs in order to see whether the tricolor and bicolor conditions described by Galton for Basset hounds could be brought in line with modern Mendelian interpretation. According to his recent paper, Castle was led to study the same problem from the same point of view. He has published a brief and important statement summarizing his results.

Our work was begun in 1908 and has gone on steadily, but slowly, since then, until a contagious disease destroyed the stock. It soon became evident that the problem is one of extreme complexity, and for its complete solution a much more elaborate and better planned series of experiments will be necessary. We hope that our results, fragmentary though they be, may serve to put on record the actual facts observed and that certain provisional suggestions that are made will be further tested.

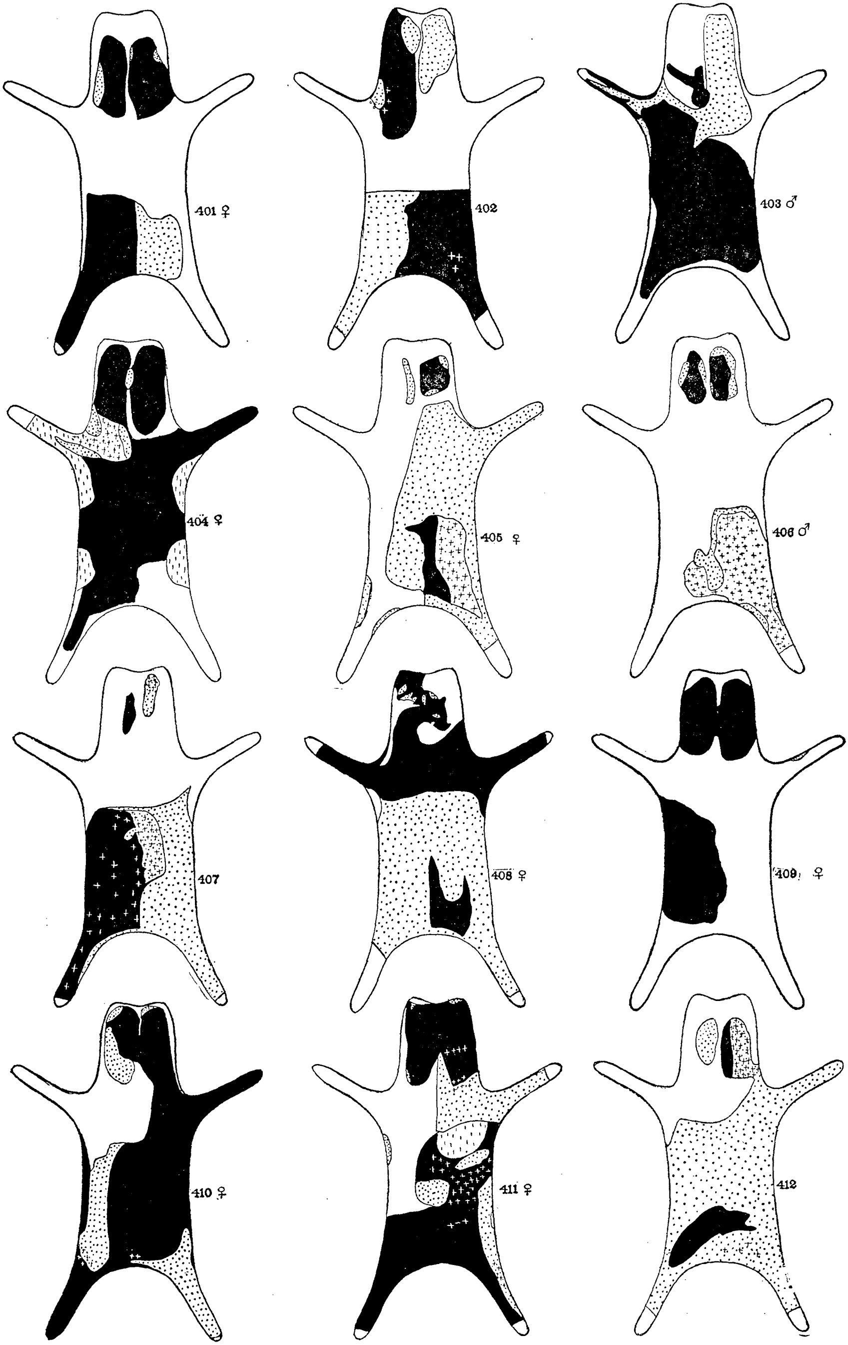
The inheritance of color in guinea-pigs has been extensively studied by Castle. Animals with a coat of uniform color may be agouti, black, yellow (red) or albino. We are concerned here only with black, red and white (not necessarily albino). When black guinea-pigs are crossed to red ones the offspring are black, or black with traces of red. Castle points out that the F_1 black is not so dark as in the pure black strain, but shows evi-

dence of the red. He states that the development of black does not hinder the development of some red pigment also in the hybrid, but the red so developed is concealed by the black. Black he regards as epistatic to red. Castle states in his recent book (1912) that in the F_2 generation three blacks to one red are produced.

Spotted animals contain white in patches. These patches may be very small in extent, or, at the other extreme, extend over the whole coat so that the eyes alone have dark pigment. These black-eyed whites, however, do not breed true, but produce spotted offspring, the spotting being variable. Black-eyed white mice give this result, and are to be sharply separated from albinos that have pink eyes and white hair. Albino guinea-pigs often have small patches of black, especially on the feet and ears, but this is not true for albino mice or rats.

In guinea-pigs the spotted animals may be black and white; or red and white. These races are said to breed true, or at least *certain* bicolor races of these kinds breed true. In addition there are races having red, black and white in their coats. These are the tricolors and it is with this race that we are here chiefly concerned. It has just been said that the tricolor is a distinct race, but this must not be understood to mean that they do not produce bicolor animals. In fact, amongst the offspring, bicolor animals continually crop out. It is this fact that has led Castle in his recent article to state that tricolors do not breed true. The bicolors produced in this way seem to differ from the pure races of bicolor in that they may produce tricolors again. For the present the question may be left open whether pure races of bicolors could be produced by selection of bicolors thrown by tricolor parents. Of course, if bicolor races had originally been incrossed, such a separation would be expected. In our experiments, at least, some bicolor individuals have appeared that seem to breed true, although the experiments are not extensive enough to settle the question.

In the following account, therefore, it should be under-


401 ♀
402
403 ♂
404 ♀
405 ♀
406 ♂
407
408 ♀
409 ♀
410 ♀
411 ♀
412

stood that when we speak of bicolored types we refer simply to the somatic character, and, as stated provisionally, we shall rank all of our bicolors, genetically, as tricolors.

Our chief problem resolves itself, therefore, into the question of how the different types of tricolor behave when mated to each other.

METHODS

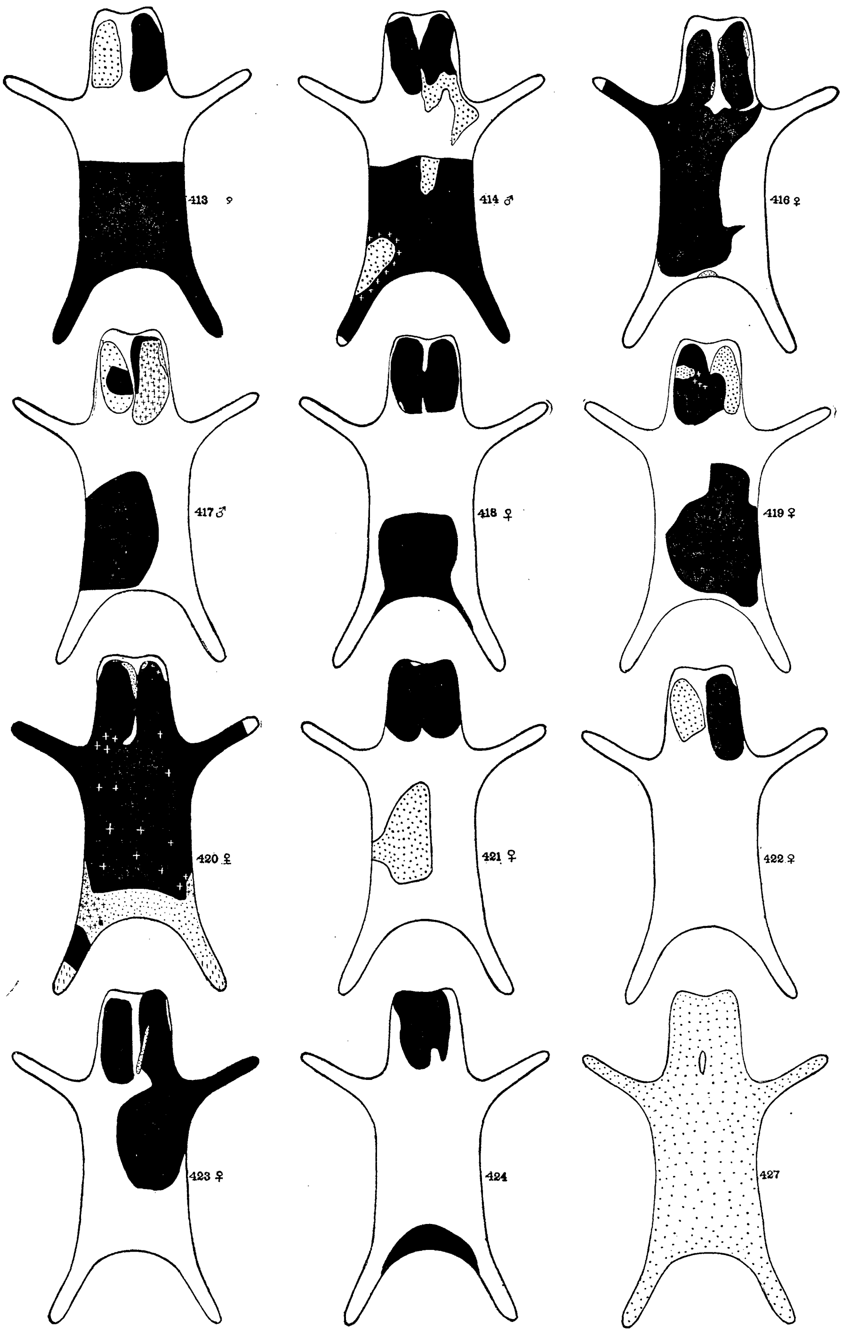
The following methods were used in these experiments:

Marking.—At first the guinea-pigs were marked by means of a numbered aluminum disk attached to the ear with wire staple. This method was unsatisfactory, as the tags were frequently torn off and lost. A system of ear holes was substituted, but this method had the disadvantage that the holes sometimes heal up in young animals. We know, however, of no better method.

Records.—The young animals were each given a number taken consecutively, and opposite the first individual of each litter the mother's and father's number was recorded together with the date of birth. A journal was also kept in which the records of the various matings were kept.

Matings.—As a rule several females were mated simultaneously with a single male. In the early part of the work the mothers were allowed to litter in the common pen and the mother identified by the presence of milk in her breasts. Sometimes two litters resulted at the same time, in which case it was impossible to assign the young to the proper mother. To avoid this, if more than one female seemed likely to litter at the same time, the females were isolated until after they had littered.

Charts.—A young individual was killed and skinned and the skin stretched just enough to hold it flat and then dried. From this a cardboard pattern was prepared and the outlines of all the sketches drawn from this. The midline of the sketch was divided into six equal parts, as an aid in locating the areas, and the various areas of


413 ♀
414 ♂
416 ♀
417 ♂
418 ♀
419 ♀
420 ♀
421 ♀
422 ♀
423 ♀
424
427

the skin drawn on the outline in free hand.[4] The majority of the sketches were made from animals which had been preserved in formalin, sometimes in poor condition when put into the formalin. A few of the dead animals were lost by being thrown out by the attendant while cleaning.

The Material.—Our original tricolors were purchased from a dealer. It is important to note that in these animals the color that we designate as red is a red and not a yellow. Animals that are spotted black, white and yellow also occur. The self-colored red and black animals were from the pedigreed stock of Mr. B. B. Horton, to whom we are under many obligations for the opportunity to carry on this work at "Oakwood." The tricolors are known to fanciers as tortoise and white.

In the figures solid black represent black; stippled areas represent yellow; white crosses on black represent yellow hairs; and black crosses represent black hairs. Small circles indicate agouti areas.

Breeding Records

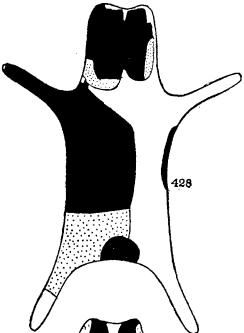
The breeding began with 401♀ (short-haired) and 402♂ (long-haired with rosettes). No. 401 we classify as tricolor black (see diagram).[5] She is the original female from which all the stock has descended. No. 402 also is tricolor (see diagram), but the black and red areas are nearly evenly balanced.[6]

The offspring from this pair are numbered from 403 to 414, inclusive; five, 405, 406, 407 (balanced), 408, 412,

[4] The presence of a few scattered white hairs on the toes has been disregarded in classifying the animal as well as preparing the sketches. Ear color also has not been considered except for bicolor black, and then only when a patch of red was present here but not elsewhere on the body.

[5] After the pedigree chart was made the individual figures of the guinea-pigs were more carefully compared and in a few cases, in which the classification was doubtful (such, for instance, as whether a pattern in tricolor black is tricolor red) was changed; the designation in the text is to be preferred to that in the table in case of disagreement.

[6] Note that 401 and 402 are partially reversed as to color areas.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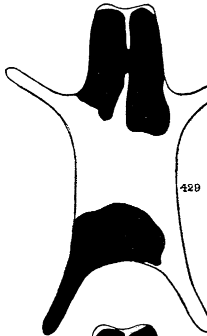
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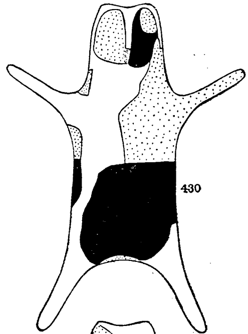
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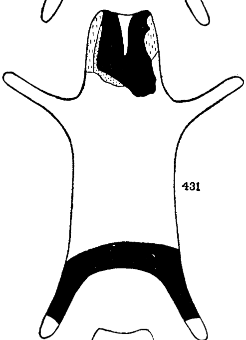
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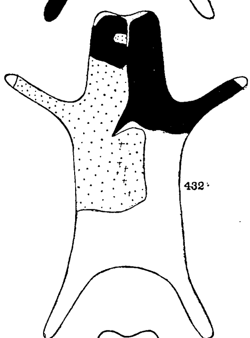
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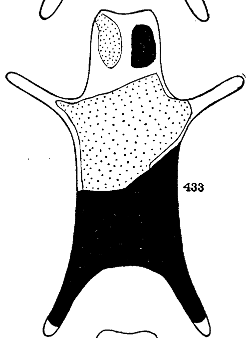
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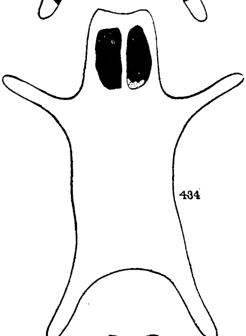
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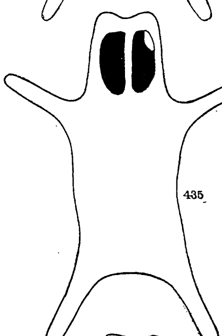
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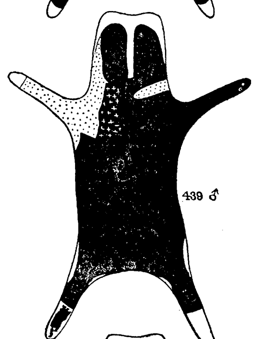
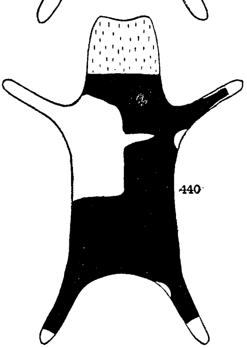

439 ♂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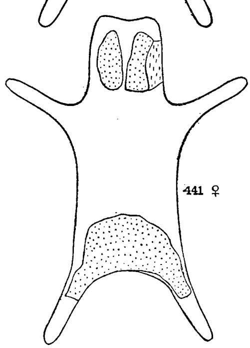
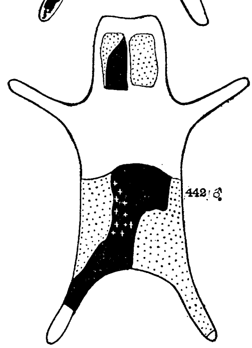

441 ♀


442 ♂

are classified as tricolor reds; 403, 404, 410, 411, 413, 414,[7] are classified as tricolor black, while No. 409 is classified as bicolor black.

Of these offspring only one is classified as bicolor, and she (409) has a trace of red on her right front leg (see diagram).

The next step was to mate, *inter se,* the tricolor blacks and the tricolor reds. For instance, 410 ♀ mated to 414 ♂ (both tricolor black) gave five tricolor blacks, 464, 465, 466, 507, 508, and three bicolor blacks—463, 475, 476. It is clear, in this instance, that tricolor blacks tended to produce the same color, *i. e.,* tricolor blacks.

Again, tricolor black 403 ♂ and 404 ♀ (she may be *413*), gave three tricolor blacks, 419, 420 and 428, and three intermediates, 421, 422 and 430, and four bicolor blacks, 418, 423, 424, 429. Four of these bicolor blacks have a trace of red.

No. 414 ♂ bred to 413 ♀ produced eight tricolor blacks: 452, 453, 490, 491, 439, 514, 515, 517; one intermediate, 516; one bicolor black, 513; two tricolor reds, 488 and 489. In this case the tricolor blacks gave two tricolor reds.

Tricolor Red[8]

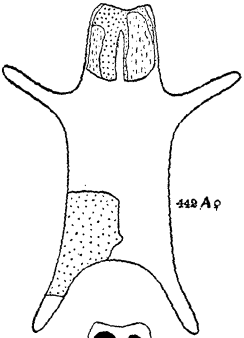
Tricolor red 408 ♀ by 406 ♂ gave one intermediate, 442; two bicolor reds, 441 and 442 A. There was present with this female at birth of the next litter, another, viz., 412 ♂, which, however, probably was not concerned in its parentage. The offspring were one tricolor black, 493; two bicolor black, 494, 495, and one tricolor red, 492.

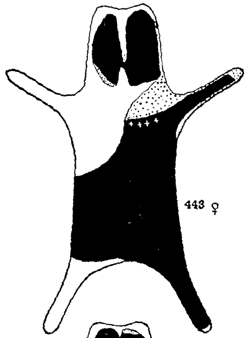
Bicolor Blacks

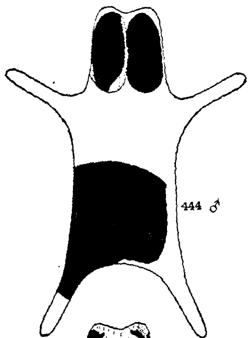
No. 409 ♀ (note red on leg) was mated to 520 ♂ and gave one intermediate, 554; two bicolor blacks, 552, 553 (reddish spot on left shoulder).

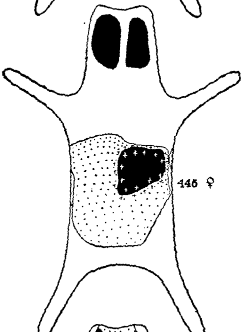
[7] The labels of 404, 410 and 413 were lost and thus the diagrams confused with one another, but not as to their parentage.

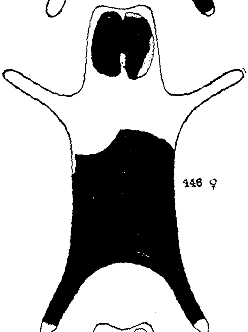
[8] Many of the tricolor reds contained much white and thus may have had more potential black, lying beneath the white, than was patent.

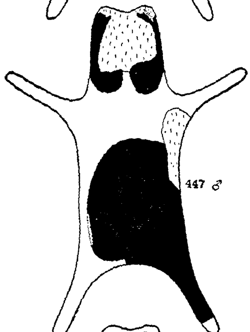

442 A ♀

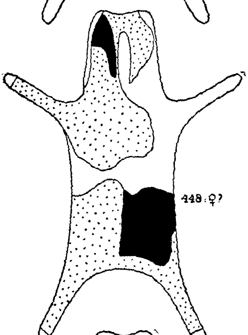

443 ♀

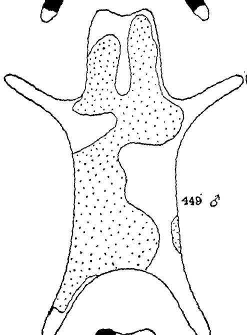

444 ♂

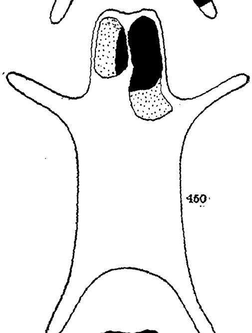

445 ♀


446 ♀


447 ♂


448 ♀?


449 ♂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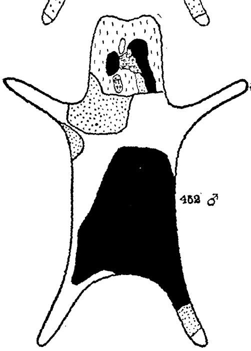
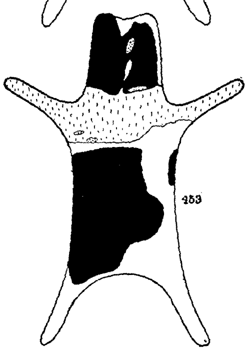

452 ♂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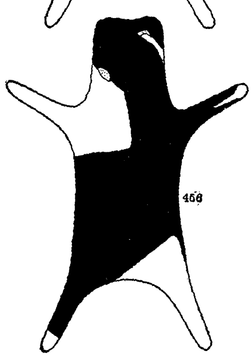
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No. 409 ♀ mated to 503 ♂ gave one bicolor black, 533, and one tricolor black, 534. We were unable to breed the bicolor reds *inter se* because of the lack of an adult bicolor red male of this stock.

Second Generation Crosses

There were no matings of tricolor blacks in this generation. A tricolor red, black-cross, was made between 489 ♀ by 492 ♂ (son) which gave bicolor red, 509, 510, 511. No more offspring could be obtained.

No. 423 ♀ bicolor black by tricolor black 469 ♂ gave tricolor black, 504, 521, 522, and bicolor black, 503 and 520.

Conclusions

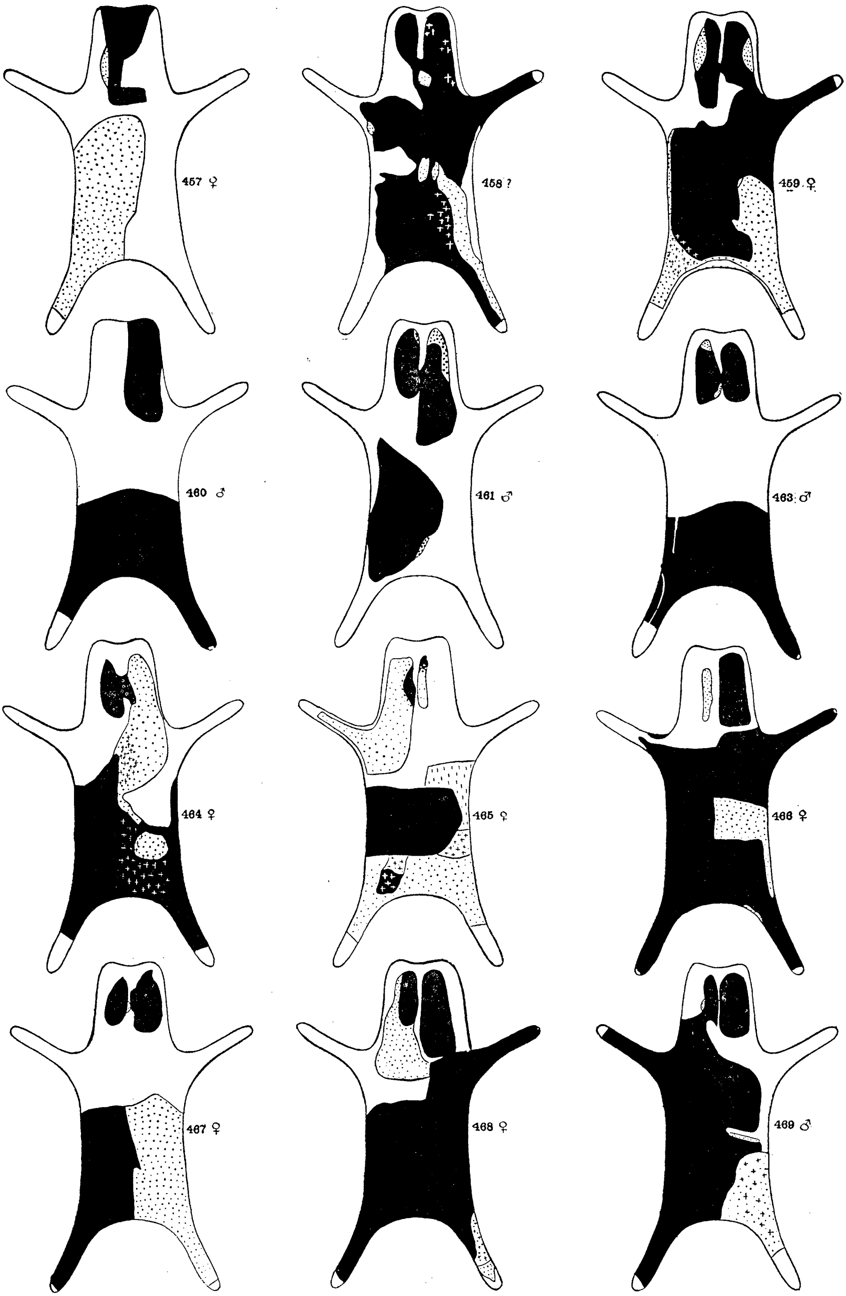
Tricolor blacks, *inter se,* gave a large number (21) of their own kind, a large number (14) of bicolor blacks, while 9 out of the 46 were either tricolor reds or intermediate; there were no bicolor reds. The two remaining individuals were classed as bicolor black, but may almost as well be called tricolor.

On the other hand, the tricolor reds mated, *inter se,* produced in 20 individuals all four classes, viz., two tricolor reds, two tricolor blacks, four intermediates, six bicolor reds and six bicolor blacks. The bicolor blacks bred, *inter se,* produced three tricolor blacks, one intermediate, eleven bicolor blacks, one tricolor red and one individual belonging to the black series, whose classification as bicolor or tricolor is uncertain. Selection for blacks gave more blacks, but the selection for red was inconclusive.

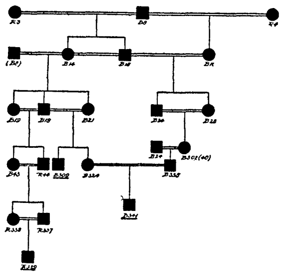
Spotted to Uniform Coat

The original tricolor black female, No. 401, was mated to a red male from Horton's stock and gave seven uniform reds, 425, 426, 427 (note white spot), 436, 437, 454 (minute spot of white on nose), 455.

One pair of these F_1 reds was mated (lack of females preventing mating more). From this pair we obtained uniform reds, 483, 484, 495, 498, 528, 594, 595, and bi-


457 ♀
458 ?
459 ♀
460 ♂
461 ♂
463 ♂
464 ♀
465 ♀
466 ♀
467 ♀
468 ♀
469 ♂

color reds[9] (mainly red), 482, 496, 497, 527, 596, and one individual, 526, much like the bicolor reds, but with a minute spot of black. It is noteworthy that although



Pedigree of "uniform" blacks and reds used in matings described in text. They came from Mr. Horton's stock.

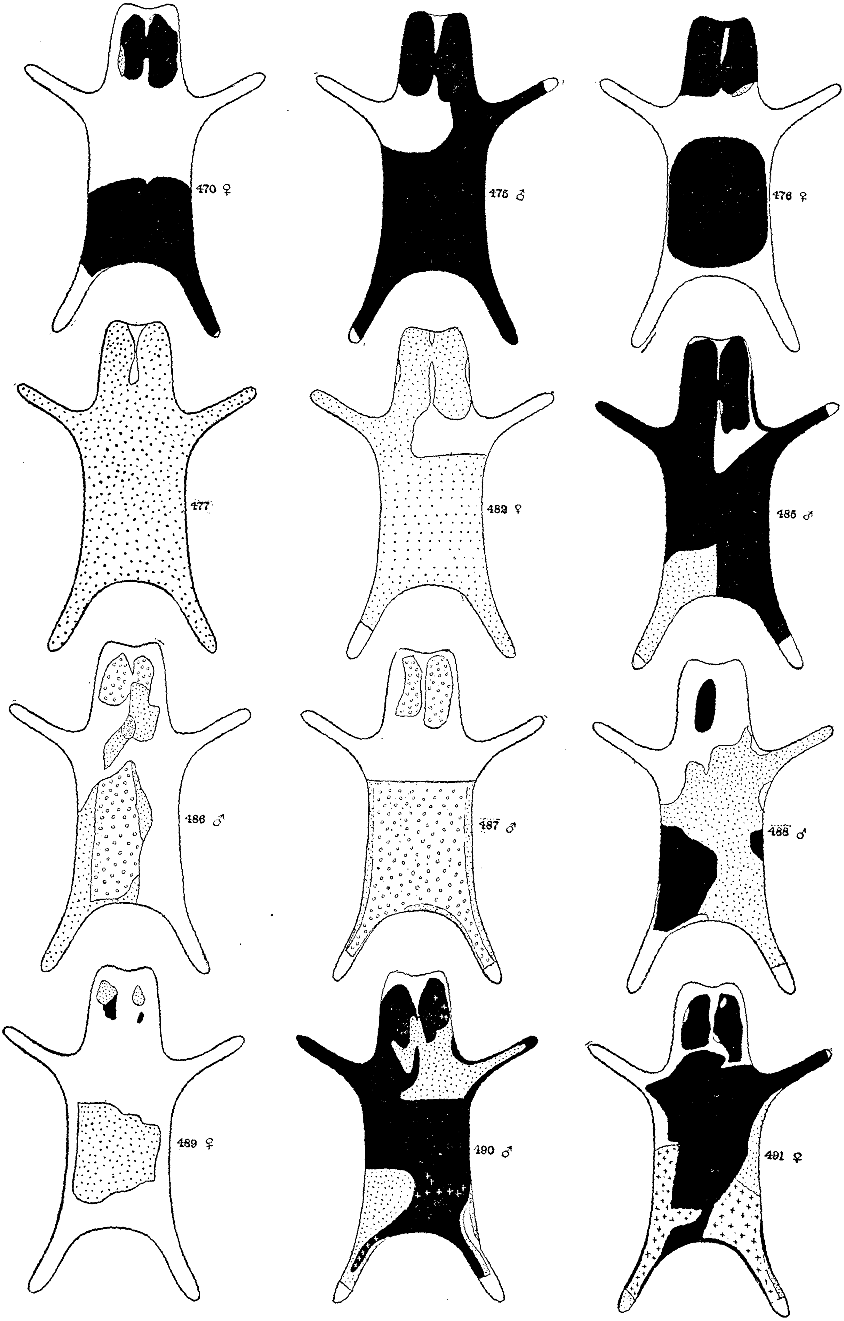
black entered into the original cross from one side it was not recovered except for the small spot of black on 526. Yet uniform black is described as dominant to red. If 401 was heterozygous for the black factor (as a single factor) black would not necessarily be expected. Only against this view is the fact that her matings with tricolor did not indicate this, and the small black spot on 526 could not be explained if this assumption were true.

One back-cross between 526 ♀ with her father, 427 ♂, gave one bicolor red, 576; and 577 (red, partially destroyed when found) and 578, classified as red.

On the other hand, when tricolor black 401 ♀ was mated to self black, 309 (Horton's stock), one young was produced, a self-colored black. In this case also uniform dominates, but the color is black.

No. 401 ♀ was also bred to another black male, and produced one black, 544, one red, 545, and one tortoise, 546. This male appears to have been homozygous as regards lack of spotted white, heterozygous for black (*B b*), and also heterozygous for some factor that causes black to

[9] On the whole the bicolor reds produced in F_2 when uniform was crossed in, had less white than the bicolor reds extracted from the tricolor series, and the white tends to occur on the anterior portion of the body.


470 ♀
475 ♂
476 ♀
477
482 ♀
485 ♂
486 ♂
487 ♂
488 ♂
489 ♀
490 ♂
491 ♀

appear in spots, *i. e.*, a factor analogous to the factor commonly recognized as the white spotting factor.

In the following crosses some tortoise colors appeared. A tortoise is black-and-red with no white.

Tricolor black, 410 ♀, by red, 339 ♂, gave four tortoise, viz., 536, 537, 538, 539.

Bicolor black, 423 ♀, by red, 339 ♂, gave two tortoise, 540 (with white blaze—not in figure) and 541.

Tricolor black, 413 ♀, by a self-black, 341 ♂, gave one uniform red.

Tricolor black, 491 ♀, by same, 341 ♂, gave black, 551.

Tricolor black, 513 ♀ (nearly bicolor black), by same male, gave uniform black, 590, and uniform red, 591.

Black, 471 ♀ (out of 401 ♀, by 309 ♂), by father, 309 ♂, gave black, 518 and 519. Later when mated to another self black, 341 ♂, she gave red, 547, 548; black, 549.

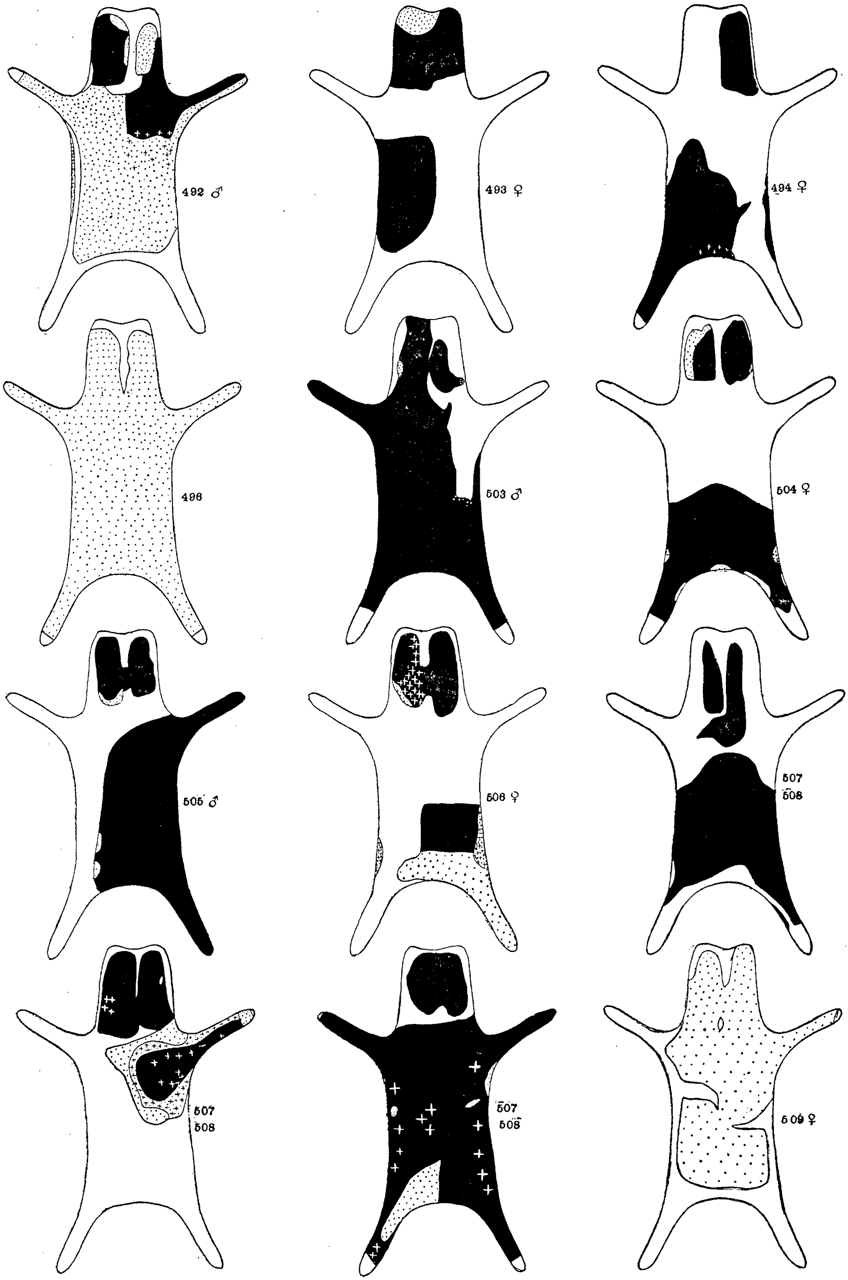
Bicolor red, 509 ♀ (nearly red?), by bicolor black, 535 (nearly black), gave tricolor black, 581, and tricolor intermediate, 532 ♂. These two opposite bicolors gave tricolors and so far as 502 is concerned two animals almost completely pigmented over the posterior half of the body produced a young that was white in these parts. Similar relations might have been pointed out in the other crosses; but reverse cases also occur.

Tortoise Inter Se, Etc.

Tortoise, 538 ♀ and 536 ♀, by 537 ♂, gave two bicolor reds, 583, 580; two uniform red, 584, 585. It would appear that tortoise, while not showing white may carry it in the same way in which a uniform animal may carry it. It is also striking that the result is like that obtained in F_2 from the mating of 401 tricolor black, to self-color red, although the F_1 is somatically very different.

Tortoise, 524 ♀, by uniform black, 341 ♂ (never crossed with spotted animals as far as known), gave tortoise, 566, and red, 567 (not on charts).

Tortoise, 524, by uniform black, 341, gave bicolor black, 587 (almost uniform), and two tortoise, 588 and 589.


492 ♂
493 ♀
494 ♀
496
503 ♂
504 ♀
505 ♂
506 ♀
507
508
507
508
507
508
509 ♀

In Castle's paper of 1905 he gives the result of certain matings between black-red and black-red. Combining the result from two tables (pages 34 and 36) there is a total of 20 black-reds and 9 reds. It may be doubted whether Castle's black-reds are always the same as our tortoise, because he speaks in the text (page 32) of a reddish-black (1,179) and (1,180), but in the table stamps them as black. One parent of these animals was black; the other red. Therefore, his "black-reds" themselves were heterozygous.

Matings of 427 ♂

This animal is one of the red offspring (except for partial blaze), out of 401, tricolor black ♀, by 201 ♂ uniform red. He was extensively mated. His offspring, by his sister, have been already described.

No. 427 ♂, mated to tricolor black, 410 ♀, gave tricolor black, 558, tricolor red, 593, tortoise, 557 (white foot), 559 (had white hind toes), 592 (nearly black).

No. 427 ♂, mated to tricolor black, 522 ♀, gave tortoise, 574, and tricolor black, 575 (not charted).

No. 427 ♂, mated to tricolor red, 489, gave uniform red, 568, tortoise, 569 (note extension of black), and tricolor black, 570 (not drawn).

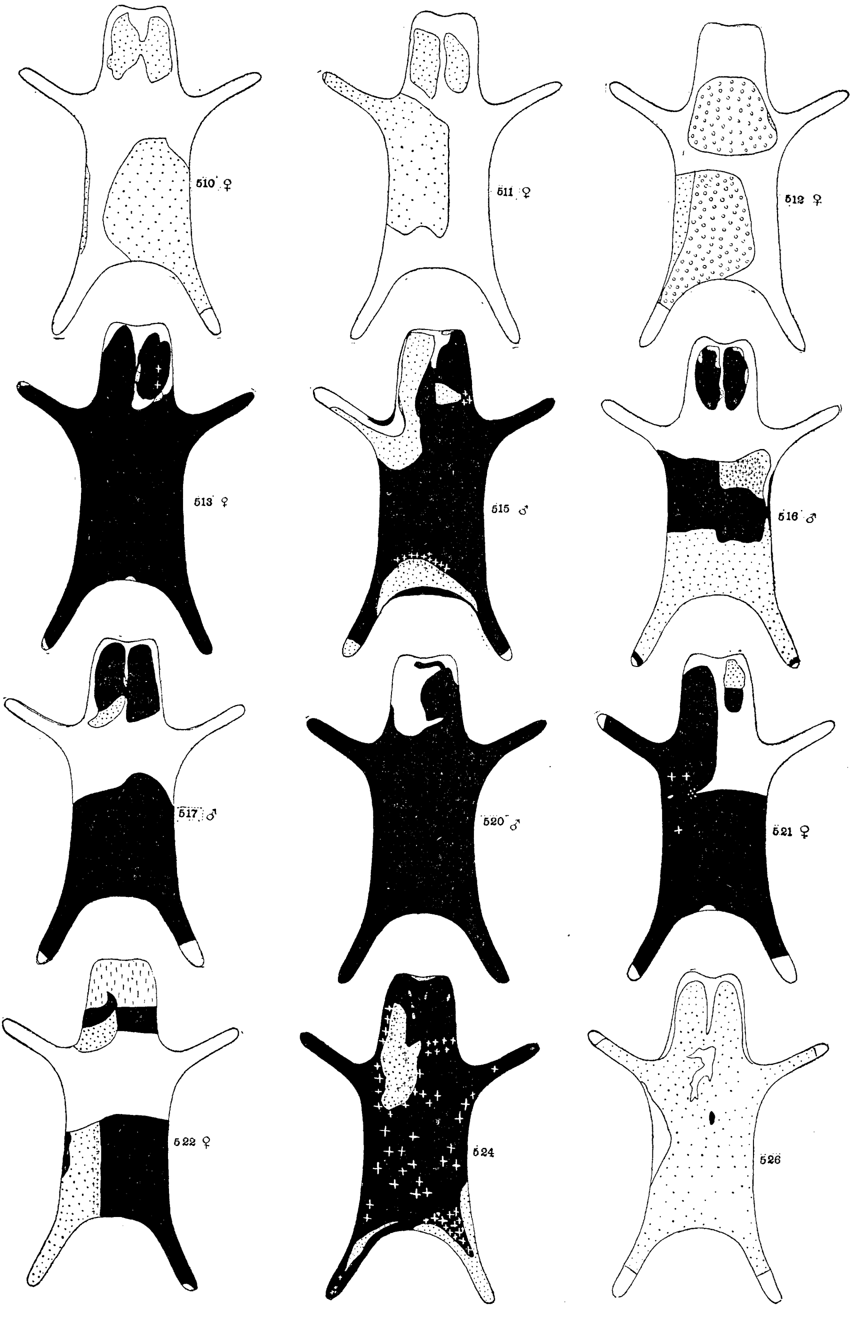
No. 427 ♂ was mated to three sisters, all bicolor reds, but not closely related to 427, viz., 509, 510 and 511. With 509 he gave uniform red, 562, 561 (not charted) and bicolor red, 562.

With 510 he gave uniform red, 563, 565, 565 *A*, and bicolor red, 564.

With 511 he gave uniform red, 571, 572, and bicolor red, 573.

Evidently 427 is heterozygous for uniform and carries no black. But when mated to black spotted, viz., 489, etc., he gave black spotted offspring.

No. 427 ♂, mated with bicolor black, 423, gave four tortoise, 579, 580, 597 and 598.



510 ♀
511 ♀
512 ♀
513 ♀
515 ♂
516 ♂
517 ♂
520 ♂
521 ♀
522 ♀
524
526

Agouti Spotted with White by Tricolors

A spotted agouti ♀ mated to tricolor black, 414 ♂, gave three tricolor blacks, 385, 499, 487, and two bicolor reds with agouti spots (*i. e.,* they had white spots, red spots and agouti spots), viz., 486, 487 and one, viz., 500, spotted agouti. It may seem that when agouti spots are present they take the place of the black. Castle's (1905) records support this suggestion. The agouti female seems to have been heterozygous for the agouti factor.[10]

DISCUSSION

It has been stated by Castle that when guinea-pigs with uniform coat are crossed to spotted guinea-pigs the offspring have uniform coats.[1] Our own limited experience confirms this statement. In the F_2 generation a variable offspring is obtained, ranging from uniform to much spotted. This question will be considered later.

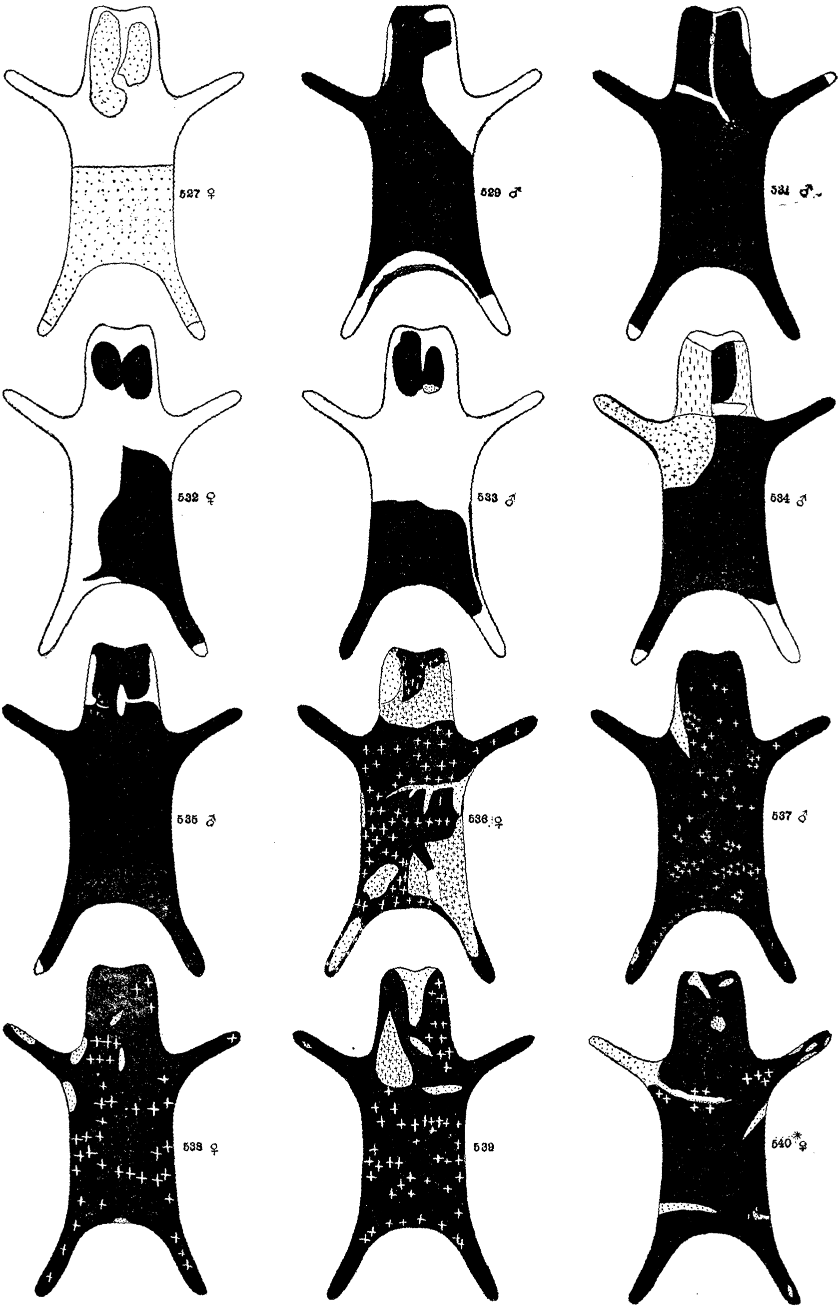
A question of fundamental importance is whether the uniform coat can be treated as allelomorphic to spotted coat. This involves the question whether spotting is the product of one factor or of more than one.

In rats and in mice the same question has come up and Cuénot has handled the problem on the basis of a pair of allelomorphs. The main evidence on which the assumption of a pair of allelomorphs rests, is derived from the number of kinds of offspring in the F_2 generation. If uniform coat is treated as allelomorphic to spotted coat, the F_2 expectation is three uniform to one spotted, and this condition is the reported result for this generation.

On the other hand, if the spotted coat is due to more than one factor the situation becomes complicated, and the F_2 expectation is no longer three to one, unless we

[10] One of the progeny of the mating of tricolor blacks, 469 ♂, by bicolor black, 423, calls for special attention. This individual, TB 521, had among the other pigmented hairs a great many that had a reddish base and a black tip. These recall, but are not identical with, agouti hairs.

[1] Castle's mating shows in some cases apparent exceptions to the rule, but possibly the uniform animals were not entirely homozygous. Exceptionally a blaze may appear in the F_1's.


527 ♀
529 ♂
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assume that there is one factor whose "absence" makes possible the development of the spotted coat. It seems to us that the experimental evidence, more especially the selection experiments of Cuénot and of Castle, suggest the possibility that the "spotted coat" is a very complex affair, depending presumably on a number of factors.

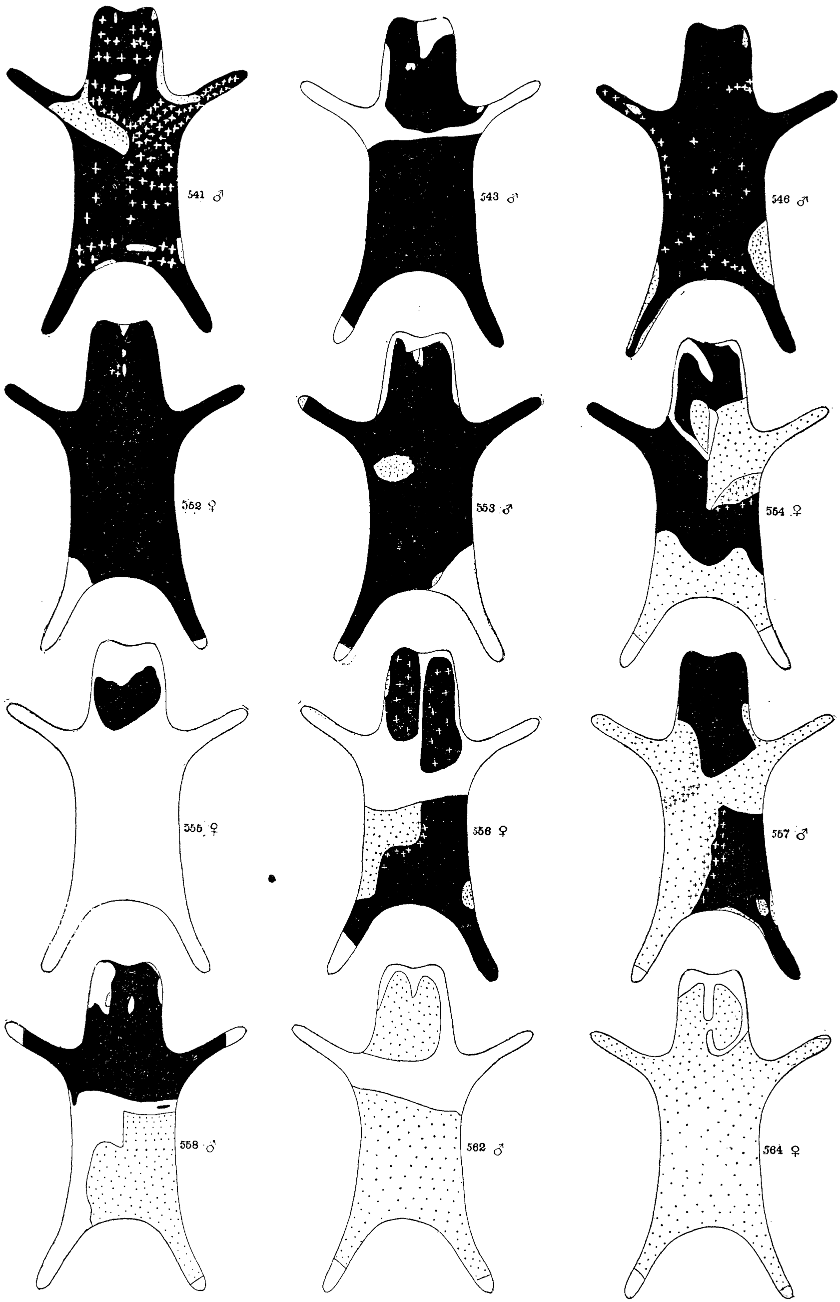
Although this possibility has been repudiated by Castle and not considered by Cuénot, it may be at least worth serious examination; for, if it should prove true, an entirely different appearance will be given to the selection experiments referred to above. Now, the fact that the modal class changes when much spotted (with white) and little spotted (with white) animals are selected, and the fact stated by Cuénot that much spotted behaves like a dominant to little spotted, suggests that we may be dealing here with a mixed population that may be treated in conformity with a Mendelian interpretation of the problem.

If much spotting has arisen through a series of progressive mutations, the following hypothesis may serve at least to put the facts in a new light.

It may be expresed in a general way as follows: If one special condition must be realized before any spotting can occur (the first realized stage may be simply due to a recessive spotting factor ss. Such an animal, mated to pure uniforms, will give:

$$
\begin{array}{ll|l}
S & S & \text{Uniform} \\
s & s & \text{Spotted} \\
\cline{1-2}
S & s & F_1 ♀ \\
S & s & F_1 ♂ \\
\cline{1-2}
SS & Ss & \\
 & Ss - ss & \\
\cline{1-2}
\multicolumn{2}{l}{1SS - 2Ss - 1ss} & F_2
\end{array}
$$

which is the simple Mendelian ratio of 3:1. In other words, the first realized stage of the spotted is a modification of the original factor and therefore its allelomorph. This means that in all ss animals the spotted


541 ♂
543 ♂
546 ♂
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554 ♀
555 ♀
556 ♀
557 ♂
558 ♂
562 ♂
564 ♀

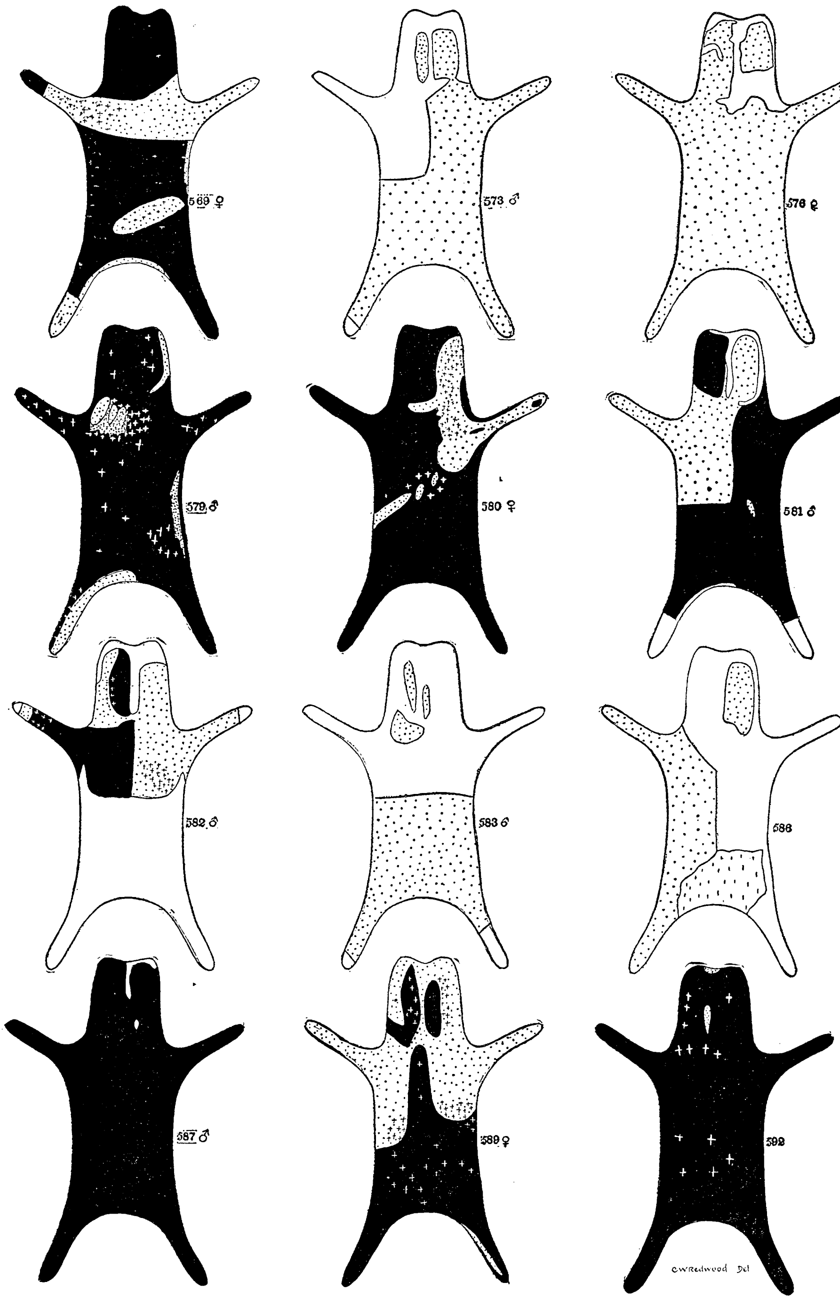
condition appears, its extent being determined by other factors.

The extension of spotting would be considered as due to successive mutations which could only be *realized* after the first stage ss had occurred. Such stages would be represented by sss_1s_1, sss_2s_2, sss_3s_3 or complexes of these, namely, $sss_1s_1s_3s_3$ or $ssS_1s_1S_3s_3$, etc. Selection would then consist in eliminating from such combinations different factors. The hypothesis is in a sense complex, but so are the facts. We shall consider this hypothesis later after our facts have been presented.

Castle has recently pointed out that there are cases of yellow-and-white-spotted guinea-pigs that breed true. In these he assumes that a chromogen factor (the one that makes any color possible) is irregularly distributed. Hence, wherever color is produced that color is yellow. Where no color is produced, because of the absence of the color producer, white results. Black-and-white races, if such exist (Castle does not specifically mention such races except black-and-white from tricolors of the tricolor series), would fall under a similar scheme. Yellow-and-black animals also exist with no white (Castle). In this case the color factor for *black* is assumed to be distributed irregularly.

Castle's explanation for the tricolors is as follows:

> Now the tricolor race is a yellow one spotted both with white and with black, *i. e.,* it results from irregularity in distribution through the coat of two different chemical substances, the color factor and the black factor. These two factors are known to be independent of each other in heredity. See Castle (1909). It is therefore not to be supposed that they will commonly coincide in distribution. If the black factor extends over all the colored areas, the animal will be black-and-white. If the black factor falls only on areas which lack the color factor, it will produce no visible effect, and the animal will be yellow-and-white. If, finally, the black factor falls on some of the colored areas but not on all of them, those in which it falls will be black, the others yellow, and the uncolored areas of course white. Hence a tricolor will result. But the gametic composition of these tricolors will not be different from that of the black-and-whites or red-and-whites produced by the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589 ♀
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CWRedwood Del

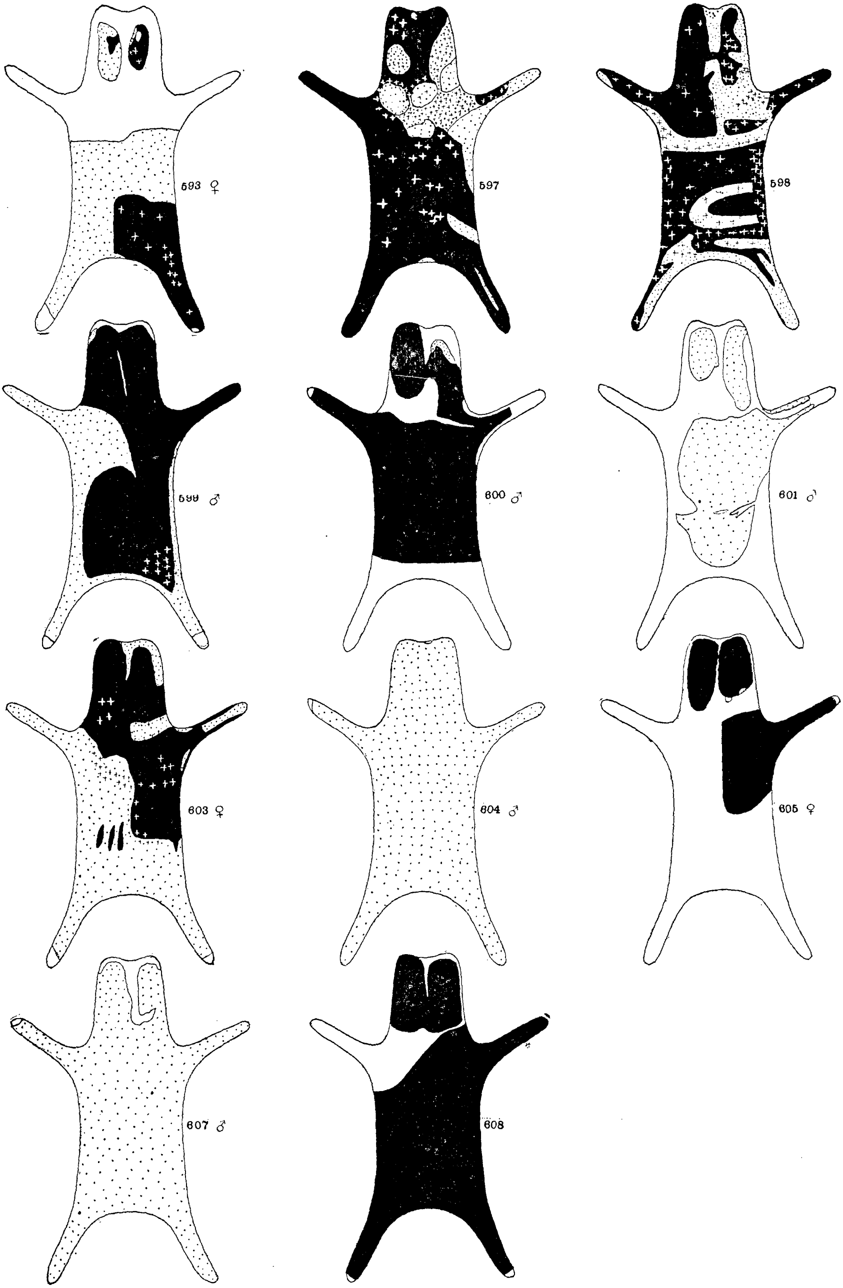
same race, since all alike will be characterized by irregularity in distribution of the same two factors A tricolor race on this hypothesis should be unfixable, as has up to the present time been found to be true.

It will be observed that this hypothesis rests on the fact that two characters are irregularly distributed, viz., black and white, and on the assumption that yellow is always uniformly distributed. What is meant by irregularity in the distribution of a character except as a statement of a fact is not clear. The words suggest somatic distribution of *factors,* at least the factors for black and for white, that have come from the germ-cell. On the other hand, it may be that the heritage of every cell is like that of all the others; and regional differences give rise to difference in pigment development. But on the last view the irregularity in distribution of the character is not explained by referring it to regional differentiation, for the question is left as uncertain as before.

There may be involved, moreover, the question of the inheritance of a pattern or patterns, for, if the spots are localized, as Castle says in his earlier papers, or, at least, if *spot-areas* are present, the distribution of black and white may not be so simple a problem as indicated by the hypothesis under consideration. Furthermore, if spotting is due not to one or two, but to several factors, a further complication is present. And finally, if a given spot is black on one side of the body and its mate is yellow on the other side, even the assumption of many factors will have difficulty in explaining the results unless a somatic segregation of the factors is assumed. Until these questions have been cleared up the explanation of the inheritance of spotting is likely to remain obscure.

Hagedoorn has recently[2] pointed out that for the occurrence of spots in rabbits and in certain other animals (cats, goats), Castle's explanation may not apply. He concludes that the distribution of color in these tricolor animals must depend upon the cooperation of many fac-

[2] AMER. NAT., November, 1912.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tors. He also points out that in tricolor dogs a spot, if on the back, is black; if on the leg, yellow. If this view is correct it would seem to follow that regional differences determine the color that develops, or that somatic segregation of color factors is definite in respect to body regions.

In the case of the Norway rat, a wild gray bred to a spotted animal gives offspring that generally contain a white spot on the belly. It would seem, in this case, that the "factor" for spotting in this region of the body is dominant over the uniform coat—the other spotting factors may be recessive, and for their development depend on the *ss*-factor.[3]

So long as these questions remain on such an unsatisfactory basis we can do little more than adopt provisionally some such view as Castle's, or else describe the facts without regard to any special theory. In the following account, therefore, we shall attempt little more than a description of the results that we have obtained. Our description resolves itself, therefore, into the question of the heredity of black-and-white somatic areas. The question of whether these are overlapping areas as Castle assumes or else spot centers in which color or no color may occur, or both is left undecided. It is certain that a spot may be large or small, and, therefore, the realized pattern is variable. Possibly we may get a clearer idea of this question if we look upon the spot as a center from which color, if present, is more likely to spread, and, if we assume somatic segregation in an early stage of the embryo the extent of the spot will be a measure of the extent to which a given cell containing the color factor multiplies as compared with neighboring

[3] We may conceive of spotting factors in two ways: Each area or center may be supposed to have a representative in the germ and each of these representatives to be entirely independent of each other in inheritance. Or there might be several factors of different sorts, such that one produces a certain pattern, another a different pattern, a third factor a third pattern and so on. The overlapping of these various patterns would still produce spotted animals.

areas that have the white factor. In pigeons the dark wing-bar of some breeds may be white in other breeds, although pigment is present, elsewhere. We can not assume, of course, a pigment producer to be absent from the germ. It seems more probable that there are special color producers, which if present in the germ, and therefore in all the body cells, give a definite reaction in that region where a white band is formed. In this case there is no *localization* factor inherent as such, *i. e.*, there is no need to assume somatic segregation, but only germinal segregation of a particular special factor that is realized in a special part. The substitution of a white area for a colored one in guinea-pigs might be looked at in the same way. But the extent to which the spot develops is a more difficult and perhaps a different problem.

The most obvious objection to Castle's hypothesis of overlapping areas is the excess of bicolors recorded both in his own and in our results, assuming that no true bicolors were in the stock. An exact lap of the black area over the red (yellow) could happen only when the black spots were of the same size or larger, and occur in exactly the same places as the red area left by the distribution of white producing factor. This would be expected to happen rarely, but, as stated above, tricolors throw a considerable percentage of bicolors.

Our matings show that the distributor for black is dominant, as seen in tricolor by uniform and tortoise by uniform giving tortoise; and tricolor by tricolor giving bicolor black. On this basis our original race of tricolors must have been heterozygous for the black distributor, and hence could throw some bicolor blacks which are real bicolors, not overlapped bicolors. This explains our excess of bicolor black which belonged to both types.